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Original Research Article

Elemental characterization of Brazilian canned tuna fish using particle induced X-ray emission (PIXE)

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ABSTRACT

The elemental composition of Brazilian canned tuna fish packed in oil and brine was determined using the particle induced X-ray emission (PIXE) technique. Three different brands representative of the Brazilian market were evaluated. The metallic cans were analyzed as well. The results indicate that the canned tuna is homogeneous (average variability ~5%) inside the cans as far as elemental concentration is concerned. The data analysis reveals that the major elements present in tuna fish are Na, Cl, K, P and S with average concentration ranging from 800 to 2400 mg/kg, while Fe (14.22 mg/kg) and Zn (4.51 mg/kg) constitute trace elements. Significant variations in the elemental concentration of canned tuna across the brands were observed for most of the elements. For instance, significant higher concentrations of Mg, P, K and Zn were observed for brand P when compared to the other brands. Moreover, significant differences were observed between oil-packed and brine-packed tuna. In this case, higher elemental concentrations were obtained for oil-packed tuna. An increasing pattern in the concentration of Fe as a function of storage time was observed for two of the brands studied in this work. Finally, the results concerning the metallic cans identified two different types of cans, namely Fe-rich cans and Al-rich cans. In general, the results obtained in this work are in good agreement with previous measurements of canned tuna.

1. Introduction

Fish is well known as a healthful source of dietary proteins rich in essential amino acids, fats, vitamins and unsaturated fatty acids (omega-3) that are associated with the reduction of risk of cardiovascular diseases and neural disorders (Gladyshev et al., 2009; Jump et al., 2012). Fish is a valuable source of essential nutrients like Na, K, P and Mg, which play a key role in human health. For instance, Na participates in several physiological processes, while K is an important electrolyte that plays an important role in regulating blood pressure and in transmitting nerve impulses to muscles (Sheng, 2000). Phosphorus is an important constituent of bones and teeth and essential for the good functioning of different hormones, while Mg is important for the structure and the function of the human body and is involved in numerous metabolic reactions (Shils, 1999).

Besides macronutrients, fish constitutes a potential source of micronutrients like Fe, Mn, Zn and Cu. Indeed, iron is an essential micronutrient for humans and its deficiency on a daily dietary intake may cause anemia (Pais and Jones, 1997). Manganese is a constituent of some enzymes and is involved in a number of physiological processes (Leach and Harris, 1997). Zinc participates in numerous metabolic processes, especially those involved with the metabolism of protein, carbohydrate, fat, and alcohol (Cousins, 1996), while copper participates in the production of hemoglobin.

On the other hand, several studies have shown that the inclusion of certain fish in human diet can increase the risk of exposure to chemical contaminants. Indeed, some fish are directly exposed to water pollution and thus accumulate these contaminants (including heavy metals) in tissues like muscle and liver (Castro-González and Mendez-Armenta, 2008). For this reason, the study of trace elements and elemental composition of fresh and canned fish has gained a new impetus in the past 10 years.

The levels of trace elements in canned tuna may vary greatly according to catch location, fish size and preparation method. Several studies involving this issue have been conducted in different countries such as United States (Ikem and Egeibor, 2005; Rasmussen and Morrissey, 2007), Poland (Usydus et al., 2008), Turkey (Mol, 2011) and Iran (Ganjavi et al., 2010). The common point among these studies is the fact that they were carried out through optical-based techniques such as ICP-MS (Mol, 2011) and ICP-OES (Ikem and Egeibor, 2005) and Flame Atomic Absorption

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Spectrometry (FAAS) (Usydus et al., 2008; Tuzen and Soylak, 2007; Ganjavi et al., 2010). One of the drawbacks of these methods is that the sample preparation is somewhat complex. This problem can be overcome by techniques like PIXE (particle induced X-ray emission), which deals mainly with solid samples. PIXE is based on the induction of characteristic X-rays by energetic ions (Johansson et al., 1995). Moreover, PIXE has a truly multi-elemental capability and no prior knowledge of the elements present in the sample is required. The sensitivity of this technique (in the range of few parts per million) compares to those provided by optical-based techniques such as ICP-MS and ICP-AES (Saitoh et al., 2002). In addition, unlike optical methods, the sensitivity provided by the PIXE technique varies smoothly as a function of the atomic number.

PIXE has been widely used to characterize a great variety of materials, including foodstuff like soybeans (Medeiros et al., 2005), peanuts (Terakawa et al., 2008), wine (Kocsonya et al., 2002; dos Santos et al., 2010) and mate tea leaves (Giulian et al., 2007, 2009).

The aim of this research was to characterize canned tuna fish from Brazil through the study of the most popular brands available in the local market using the PIXE technique. In this study, tuna fish and their respective metallic cans were analyzed in order to check any possible correlation between the cans and theirs contents. Moreover, a statistical analysis was carried out in order to identify the variability of the elemental concentrations among canned tuna of different brands, moistures and date of canning. Different portions of the tuna fish inside the cans were analyzed as well, thus enabling the assessment of the homogeneity of the contents inside the metallic cans. Fresh tuna was analyzed and compared to tuna packed in cans and in sachets. Finally, the results were compared to those obtained from other studies.

2. Materials and methods

2.1. Samples

Three different Brazilian brands of canned tuna packed in brine were purchased from a local market in the city of Porto Alegre (state of Rio Grande do Sul). For each brand, tuna cans of 170 g net weight manufactured in 2007 were randomly selected from the market. It is important to note that throughout this work "*n*" refers to the number of independent samples analyzed. The brands were Gomes da Costa (G) ($n = 3$ samples), Pescador (P) ($n = 5$ samples) and Coqueiro (C) ($n = 3$ samples). Brand G constitutes the largest producer of canned tuna in Brazil. For one specific brand (C), samples of tuna canned in oil were also purchased ($n = 8$ samples). Tuna-in-oil cans were not available for brands G and P.

According to manufacturers, the following tuna species are used in the Brazilian tuna canning industry: Skipjack tuna (*Katsuwonus pelamis*) and Yellowfin tuna (*Thunnus albacores*) for brand G; Skipjack tuna, Albacore tuna (*Thunnus alalunga*), Yellowfin tuna, Blackfin tuna (*Thunnus atlanticus*), Bigeye tuna (*Thunnus obesus*), Southern Bluefin tuna (*Thunnus maccoyii*), Atlantic Bluefin tuna (*Thunnus thynnus*) and Longtail tuna (*Thunnus tonggol*) for brand P; and Skipjack tuna, Yellowfin tuna and Bigeye tuna for brand C. It is important to bear in mind that the fish species used in canning may vary according to the season and place they are caught.

Prior to preparing the samples, the external parts of the cans were thoroughly washed and cleaned with a neutral detergent (phosphate-free) and carefully rinsed with water. After opening, the moisture (brine or oil) was carefully drained from the cans.

In order to check the elemental homogeneity of the tuna inside each can, the tuna was sub-sampled in three parts: (a) the upper portion which was in contact with the lid ($n = 2$ samples); (b) the inner portion which was not in contact with the metallic parts of the can ($n = 3$ samples); (c) the lower portion which was in contact with the bottom of the can ($n = 3$ samples). Since the PIXE system requires the use of solid samples, the contents of each can had to be dried up. To that end, samples were weighed and put in a beaker inside an oven at relative low temperature (70 °C) for 2 h, thus ensuring an adiabatic thermal procedure. Once dried, the samples were homogenized and pressed into pellets of 25 mm of diameter and 2 mm thick. Eight pellets were obtained for the contents of each of the 19 cans analyzed, totaling 152 pellets.

The effects of packing on tuna fish were studied by analyzing tuna fish from brand C packed in cans and sachets. For both cases the moisture was oil. They were manufactured at the same time and the packages were stored for 30 months before opening for analysis. The sachet-packed tuna samples (brand C) were also cleaned with detergent and rinsed with water prior to opening the pack. A total of 2 packs were purchased and 4 samples were prepared.

For comparison purposes, 2 pieces of fresh tuna were purchased in different days from a local market and prepared for the PIXE measurements. In total, 5 samples of fresh tuna were prepared. For both fresh tuna and sachet-packed tuna, the samples were prepared following the same procedure used to prepare samples of canned tuna.

Finally, the metallic cans of all brands were studied as well. After opening, the cans were washed and rinsed with tap water. A visual inspection of all cans was carried out in order to check their integrity. None of them presented problems like fractures, corrosion and enamel failure, which could lead to the contamination of the tuna inside them (Kontominas et al., 2006). For each metallic can, the internal part of the lids and bottoms were analyzed. In total, 27 metallic can samples were prepared.

2.2. Instrumentation

2.2.1. PIXE

The elemental analysis was carried out at the Ion Implantation Laboratory of the Physics Institute of the Federal University of Rio Grande do Sul (UFRGS), Porto Alegre, Brazil. A 3 MV Tandetron accelerator delivered 2 MeV protons at the PIXE station for elemental analysis. The average current was 3 nA. The chamber is completely insulated from the accelerator and therefore was used as a Faraday cup in order to obtain the total charge for each experiment. The samples (tuna pellets and metallic cans) were loaded in a ladder-type holder capable of accommodating 10 samples at the same time. The samples were positioned in the vacuum chamber using an electromechanical system and a camera for visualization. The pressure inside the PIXE chamber was of the order of 10^{-6} mbar throughout the experiments. The X-rays induced by the proton beam were detected by a Si(Li) detector placed at 135° with respect to the beam line. The overall energy resolution of the detection system was 155 eV at 5.9 keV. In order to avoid charge buildup in the samples, an electron flood gun was used in all experiments (Shubeita et al., 2005).

In order to check the presence of trace elements such as Al, Ti, Cr, Rb, Sr, Mo, Hg and Pb, high statistics PIXE measurements were also carried out using high current (6 nA) and longer irradiation times, namely two- to three-fold higher than the average irradiation time normally used during the measurements. In this way, the counting statistics and the peak-to-background ratio were improved for those elements. Three samples from each brand were analyzed in such way.

2.2.2. RBS

The Rutherford backscattering spectrometry (RBS) technique (Chu et al., 1978) was used in order to obtain the relative concentration of light (matrix) elements like C, O and N present in the tuna samples. A 1.2 MeV He^{+} beam with an average current of

Table 1
Trace metal concentrations in certified reference materials (NRCC-DORM-2 Dogfish muscle and BCS-CRM 387/1 Nimonic 901 Alloy) and experimental recoveries.

Element	Dogfish muscle		Element	Nickel alloy	
	Certified value	Measured value		Certified Value	Measured value
Al	10.9 ± 1.7	10.6 ± 1.2	Al	2400 ± 100	2344 ± 20
Cr	34.7 ± 5.5	35.8 ± 2.4	Ti	30,000 ± 500	31,481 ± 1085
Mn	3.66 ± 0.34	3.44 ± 0.53	Cr	113,500 ± 700	118,740 ± 2330
Fe	142 ± 10	145.8 ± 7.3	Fe	384,000 ± 500	398,021 ± 4377
Ni	19.4 ± 3.1	19.9 ± 1.5	Ni	412,000 ± 2000	415,860 ± 644
Cu	2.34 ± 0.16	3.05 ± 0.5	Mo	58,300 ± 600	56,919 ± 311
Zn	25.6 ± 2.3	24.6 ± 1.5			
As	18.0 ± 1.1	18.0 ± 0.6			

All values (average ± standard deviation of $n = 10$ independent samples) are in mg/kg.

22 nA was employed in all experiments. The backscattered ions were detected by a Si surface barrier detector placed at 158° with respect to the beam direction. The pressure inside the reaction chamber was of the order of 10^{-6} mbar during the experiments. The total charge was obtained after a calibration using a rotating vane intercepting the beam periodically.

2.3. Data analysis

2.3.1. PIXE standardization procedure

The standardization procedure adopted in this work relies on the comparison of standards with the samples under study (Johansson et al., 1995). All experimental parameters like geometric factors, detector's solid angle, absorbers, beam energy and accumulated charge are taken into account. The present work made use of a dogfish muscle tissue standard (Certified Reference Material DORM-2, National Research Council Canada – NRCC) and a metallic standard (Nimonic 901 Alloy 387-1). In order to validate our results, the certified standards materials were measured several times in the same experimental conditions of the analyzed samples. Table 1 shows their certified values as well as the recovery values obtained in the experiment.

2.3.2. PIXE analysis

The PIXE spectra were analyzed using GUPIXWIN software package (Campbell et al., 2000). This software performs a least-squares fitting of all peaks present in spectrum simultaneously, converting peak areas to elemental concentrations through the use of physical parameters like ionization cross sections and ion stopping power. Moreover, this software handles the continuum background using a top-hat filter operation including discreet and continuum pileup effects. Physical processes like secondary fluorescence and self-absorption in the target are evaluated as well. As a result, each element present in the sample is assigned to a particular concentration. The final uncertainties quoted for the elemental concentrations take into account all the standards used in the standardization procedure, all the experimental parameters and the uncertainties arising from least-square fitting procedure of the X-ray spectra.

2.3.3. RBS analysis

The RBS spectra were analyzed with the SIMNRA code (Eckstin and Mayer, 1999). This software package simulates Rutherford and non-Rutherford backscattering data for particular geometries and a variety of ion-matter combinations.

2.3.4. Statistical analysis

The final concentration of a particular element was obtained through the mean arising from the analysis of several samples, while the standard deviation was taken as representative value related to the variance of the results. For the comparison among different sets of data, the ANOVA and Tukey's post hoc test (5% level of significance) were employed.

3. Results and discussion

3.1. Elemental concentration of metallic cans

The elemental concentrations of all metallic cans employed in the present study were evaluated through the PIXE technique. The elemental concentrations are shown in Fig. 1. Two distinct types of metallic cans are clearly identified. Brand P appears to employ aluminum-rich metallic cans, with important contributions of Mg, Cl, Mn and Cu. For brand P, the concentration of Al is approximately 400 times higher than the concentration of Fe. On the other hand, brands G and C make use of metallic cans rich in iron. In this case, the ratio between the elemental concentrations of Al and Fe amounts to 0.16 and 0.11 to brands G and C, respectively. One striking difference between brands G and C is the concentration of chlorine, which is much higher for brand G. Indeed, the elemental concentration of Cl found in the cans of brands G and C amounts to 3240 mg/kg and 40 mg/kg, respectively.

3.2. Elemental concentration of canned tuna

The RBS analysis revealed that the matrix of the canned tuna is compatible with 70% carbon, 15% oxygen and 15% nitrogen (dry weight).

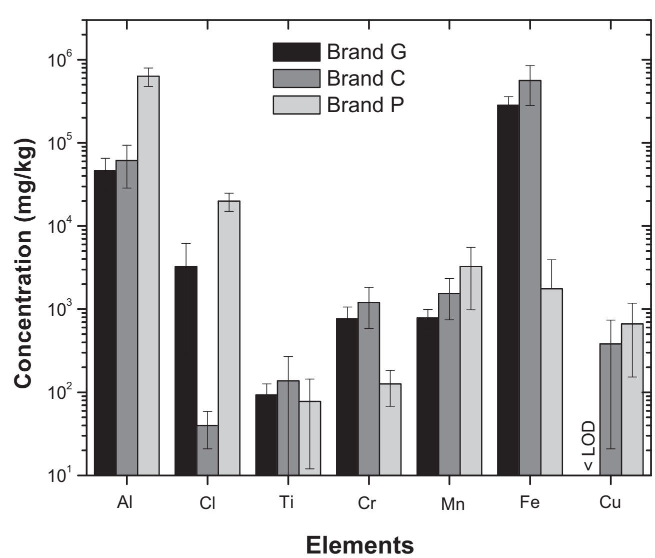

Fig. 1. Elemental concentrations of cans from different brands. The average LOD for each element, in mg/kg is: 477 for Al, 30 for Cl, 46 for Ti, 23 for Cr, 22 for Mn, 80 for Fe and 30 for Cu.

Elements such as Na, Ca, Cl, K, P, S, Mg, Fe and Zn, were simultaneously determined in all canned tuna samples through the PIXE technique. According to the statistical analysis, no significant differences (5% level of significance) were observed among the sets containing different portions of canned tuna. This result indicates that the contents of each can are homogeneous as far as elemental concentration is concerned. For instance, the average homogeneity for a trace element such as Fe and a major element such as P were found to be 6.3% and 4.3%, respectively in each can. Therefore, all samples ($n = 8$) from each can were averaged to yield the final results representative of that particular can.

In Table 2 we present the results obtained in this work for brine-packed and oil-packed canned tuna. The overall results of all canned tuna samples obtained in this study along with some other results available in the literature are shown as well. It is important to mention that the concentrations of S, Mn, Cu and Ni were below the limit of detection (LOD) in some samples. In these cases, such values were not taken into account for the averages presented in this work. Therefore, the results obtained for S, Mn, Cu and Ni were calculated with a smaller number of samples.

In general, the results show that Na and Cl have the highest concentrations (about 2000 mg/kg) followed by K and P (concentrations around 900 mg/kg). In addition, the concentrations of Mg and S were found to be in the range between 150 and 500 mg/kg. The content of Ca in canned tuna was found to be about 20 mg/kg. The concentrations of trace elements such as Fe, Mn, Ni, Cu and Zn were in the range between 1 and 15 mg/kg.

The contents of sodium and potassium observed in the present work lie in the ranges determined for the Mexican canned tuna (Castro-González et al., 1998). Like the present study, these elements were found to be the most abundant minerals in Mexican canned fish. Our results for phosphorus appear to be compatible to those determined in Polish canned tuna (Usydus et al., 2008). On the other hand, the concentration of Ca in Brazilian canned tuna is much lower (around 20 mg/kg) than the level observed by Usydus et al. (2008) and Castro-González et al. (1998). Finally, another major element found in Brazilian canned tuna is Cl. Chlorine is a major element that is essential for the formation of gastric acid and for helping in the transportation of hormones.

Concerning trace elements, our result for Fe agrees well with data obtained for American (Ikem and Egeibor, 2005) and Turkish (Tuzen and Soylak, 2007; Mol, 2011) canned tuna. Moreover, the levels of Mn observed in Brazilian canned tuna are similar to the one reported by Tuzen and Soylak (2007) and somewhat higher than the mean value observed by Ikem and Egeibor (2005). The Zn content obtained in this work is much lower than the values reported by Tuzen and Soylak (2007) and Mol (2011), but lies in the ranges reported by Ikem and Egeibor (2005) and Castro-González et al. (1998). In this case, it is important to bear in mind that all results are below the limiting values set by FAO (1983) and the Brazilian legislation (ABIA, 1998).

The concentration of Cu found in the Brazilian canned tuna is compatible with the ranges reported by Mol (2011) and Ikem and Egeibor (2005) and much lower than the value quoted by Tuzen and Soylak (2007). The level of Cu found in the present work is much lower than the limiting values established by FAO (1983) and WHO (1999). Finally, the concentration of Ni in the Brazilian canned tuna is compatible with the value observed by Tuzen and Soylak (2007) for the Turkish canned tuna.

Concerning the high statistics measurements, the overall results show that the values found for Al, Ti, Cr, Rb, Sr, Mo, Hg and Pb are either compatible with or below the LOD of the system, thus indicating relatively low concentrations of these elements in canned tuna. For example, the average concentration of Hg found in our measurements is compatible with the LOD of the system 0.80 ± 0.22 mg/kg wet weight. Other studies appear to point in the same direction concerning metals like Cd and Pb (dos Santos et al., 2009). Yallouz et al. (2001) investigated the Hg levels in five different brands of canned tuna commercialized in Rio de Janeiro. According to the results, 53% of the samples contained levels above the WHO (World Health Organization) recommended values (0.5 μg/g) (WHO, 1999). Nevertheless, it must be stressed that four out of five brands analyzed contained tuna fish imported from different countries such as Mexico, Peru, Venezuela and Thailand. The brand whose tuna fish came from Brazil had Hg content below the WHO limit value.

3.3. Comparison among canned tuna of different brands

Table 3 gives the elemental concentration of brine-packed canned tuna for the brands studied in this work. For some samples, the elemental concentrations were not significantly above the limit of detection (LOD) and therefore the LOD appears as a lower limit of the range for that particular brand. In such cases, these samples were not included in the calculations of the mean and standard deviation (sd) shown in Table 3.

According to Table 3, significant variation in the concentrations of Mg, P, K and Zn across the three brands was observed ($P < 0.05$). In this case, brand P presented the highest level of these elements. On the other hand, the levels of Na and Cl in brand C were found to be significantly lower than in brands G and P. In addition, canned

Table 2
Average elemental concentrations of Brazilian canned tuna compared to those from other major markets (mg/kg wet weight).

Element	This work, $n = 86$ (brine)	This work, n = 24 (oil)	This work, $n = 110$ (overall)	Ikem et al., $n = 29$ (oil and brine)	Tuzen et al., $n = 4$	Usydus et al., $n = 8$ (oil)	Castro-González et al., $n = 7$ (oil)	Mol, $n = 15$ (oil)
	Mean ± sd	Mean ± sd	Mean ± sd	Range (mean)	Mean ± sd	Mean ± sd	Range	Range
Na	2280 ± 937	2592 ± 369	2347 ± 855	–	–	–	1360–5520	
Mg	156 ± 52	154 ± 31	156 ± 48	–	–	–	–	–
P	897 ± 215	1035 ± 136	927 ± 208	–	–	1290 ± 176	–	–
S[a]	465 ± 198	1648 ± 110	803 ± 567	–	–	–	–	–
Cl	2049 ± 853	3016 ± 786	2264 ± 932	–	–	–	–	–
K	958 ± 253	1165 ± 227	1004 ± 261	–	–	–	780–2210	–
Ca	23.09 ± 12.17	22.64 ± 5.67	23.02 ± 11.28	–	–	473 ± 189	34–218	–
Mn[a]	0.87 ± 0.47	1.42 ± 0.44	1.02 ± 0.53	0.08–0.63 (0.22)	0.90 ± 0.08	–	–	–
Fe	15.08 ± 6.35	9.49 ± 4.46	14.22 ± 6.4	0.01–88.4 (15.8)	14.9 ± 1.1	–	–	20.2–38.7
Ni[a]	0.91 ± 0.30	ND[b]	–	0–124.5 (4.3)	0.85 ± 0.06	–	–	–
Cu[a]	0.93 ± 0.44	0.96 ± 0.27	0.94 ± 0.39	0.01–0.51 (0.25)	2.50 ± 0.12	–	–	0.48–0.58
Zn	4.72 ± 2.18	3.44 ± 1.46	4.51 ± .16	0.14–9.87 (4.78)	17.8 ± 1.2	–	2–7	8.20–12.4

n = number of independent samples analyzed.
[a] These elements were not observed in all samples analyzed.
[b] LOD: 0.82 ± 0.19.

Table 3
Range and average elemental concentrations of brine-packed canned tuna fishes from 3 different brands (mg/kg wet weight).

Element	Brand G (n = 35)		Brand P (n = 32)		Brand C (n = 19)		LOD
	Mean ± sd	Range	Mean ± sd	Range	Mean ± sd	Range	Mean ± sd
Na	2635 ± 429^{A}	1267–3874	2320 ± 1235^{A}	921–6109	1596 ± 649^{B}	776–3022	41 ± 10
Mg	142 ± 33^{A}	70–199	202 ± 45^{B}	100–290	106 ± 23^{C}	66–146	26 ± 5
P	882 ± 147^{A}	654–1231	1038 ± 223^{B}	541–1490	689 ± 95^{C}	511–911	11 ± 3
S[a]	510 ± 217^{A}	173–883	407 ± 195^{A}	156–678	456 ± 61^{A}	377–523	94 ± 50
Cl	2452 ± 402^{A}	765–3552	2076 ± 1038^{A}	792–4947	1306 ± 614^{B}	646–2589	23 ± 5
K	933 ± 211^{A}	549–1332	1142 ± 213^{B}	805–1691	705 ± 105^{C}	519–916	3.6 ± 0.9
Ca	20.8 ± 6.7^{A}	9.1–43.2	30.4 ± 15.3^{B}	16.01–82.3	15.0 ± 5.5^{A}	9.3–34.0	5.4 ± 1.1
Mn[a]	0.82 ± 0.40^{AB}	≤LOD–1.73	1.09 ± 0.55^{A}	≤LOD–2.76	0.58 ± 0.21^{B}	≤LOD–1.04	1.17 ± 0.54
Fe	16.2 ± 4.8^{A}	10.2–30.0	11.2 ± 3.5^{B}	6.1–22.6	19.6 ± 8.7^{A}	11.6–38.8	0.80 ± 0.36
Ni[a]	1.17 ± 0.30	≤LOD–1.4	0.83 ± 0.17	≤LOD–1.0	0.69 ± 0.05	≤LOD–0.76	1.15 ± 0.76
Cu[a]	1.00 ± 0.36^{AB}	≤LOD–1.57	1.21 ± 0.44^{A}	≤LOD–1.83	0.60 ± 0.23^{B}	≤LOD–1.11	1.17 ± 0.76
Zn	4.23 ± 1.33^{A}	1.65–6.71	6.50 ± 2.08^{B}	1.45–13.40	2.64 ± 0.96^{C}	0.96–5.02	1.25 ± 0.74

n = number of independent samples analyzed.
Different letters (A–C) in the same line indicate significant differences among the brands ($P < 0.05$).
[a] These elements were not observed in all samples analyzed.

tuna from brand P had significantly higher content of Ca if compared to the other brands, whereas the concentration of Fe in brand P was found to be lower than in brands G and P. Moreover, mean values for Mn and Cu in brand C were significantly lower than those of brands G and P. Finally, there was no significant variation in S across the various brands.

The differences in the concentrations of some elements observed among the brands could be explained by several factors such as fish species used by each manufacturer, processing steps, type of cans and storage conditions. From Table 3 one can obtain the standard deviation of the mean, which is associated with the variance for a particular sample size. Brand P has the highest standard deviation of the mean for all elements but S, Fe and Ni. Moreover, the concentration of Fe from this brand is the lowest among the brands. Interestingly, brand P uses a greater variety of species if compared with brands G and C. Moreover, the can used by this brand is rich in aluminum and presents relatively low concentrations of iron. Despite these observations, it is difficult to establish a definite correlation among these results due to the relatively large uncertainties associated to the data obtained in the present study.

3.4. Elemental concentration: brine × oil

An additional study was carried out with canned tuna from brand C. For this brand, tuna samples canned in brine and in oil were analyzed in order to investigate any possible differences in the elemental concentrations for different moistures. The statistical analysis shows significant differences ($P < 0.05$) for all elements but zinc. In general, oil-packed tuna presented comparatively higher levels for most elements than the brine-packed tuna. Some results are shown in Fig. 2. For instance, the S content in tuna fish packed in oil is four-fold higher than in tuna fish packed in brine. Moreover, Na, Cl and K levels in canned tuna packed in oil are approximately two-fold higher than the levels observed in canned tuna packed in brine. The only exception to this rule is Fe whose level is lower in tuna packed in oil.

Few studies have reported some data showing differences in some trace elements levels when comparing oil-packed or brine-packed fish. For instance, Tarley et al. (2001) observed that sardines canned in tomato sauce presented higher iron concentration than those canned in soybean oil. Moreover, according to Burger and Gochfeld (2004), a comparison between tuna packed in oil and in water yielded no significant difference for the concentration of mercury. However, the concentration of mercury in tuna packed in oil is slightly lower than that packed in water (Burger and Gochfeld, 2004).

3.5. Elemental concentration: oil-packed tuna × fresh tuna

Fig. 3 shows the comparison between fresh and packed tuna of brand C. Two distinct features can be observed in Fig. 3. First of all, there is no significant difference between tuna packed in sachet and tuna packed in can. Another interesting feature arises from the comparison between oil-packed tuna (sachet and can) and fresh tuna. Basically, elements like P, S, Cl, K and Ca have a much lower concentration in fresh tuna. This result suggests that other factors like manufacturing process and moisture (oil in this case) may play a significant role in the elemental concentration of processed tuna. However, it is important to bear in mind that the primary source of tuna (fresh and oil-packed) is not necessarily the same. Moreover, the time of catch is surely different. These differences may have an impact on the final concentrations as well.

3.6. Fe concentration of canned tuna as a function of storage period

The elemental composition of canned tuna fish was also evaluated as a function of storage period. To that end, the cans were sorted out according to the storage time. The storage time was defined as the period between the canning date and the day the cans were opened for analysis. Fig. 4 shows the evolution of the concentration of Fe as a function of the storage time for all brands.

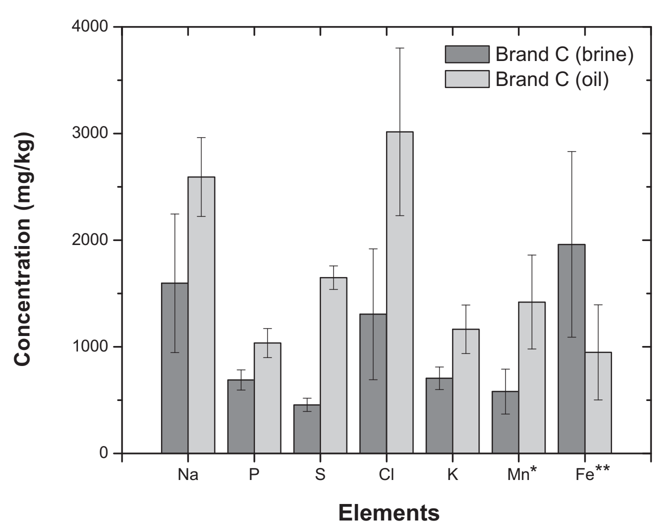

Fig. 2. Elemental concentrations of brine-packed and oil-packed tuna from brand C. The actual concentrations of Mn and Fe were multiplied by 1000 and 100 in order to facilitate their comparison with other elements in the same plot.

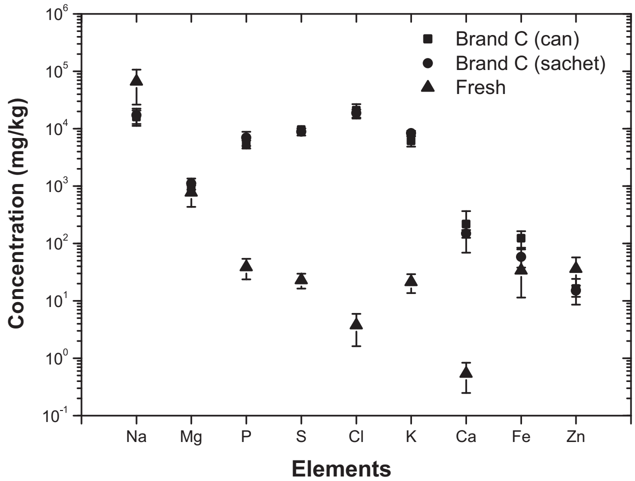

Fig. 3. Elemental concentrations of oil-packed tuna fish (from cans and sachets of brand C). and fresh tuna.

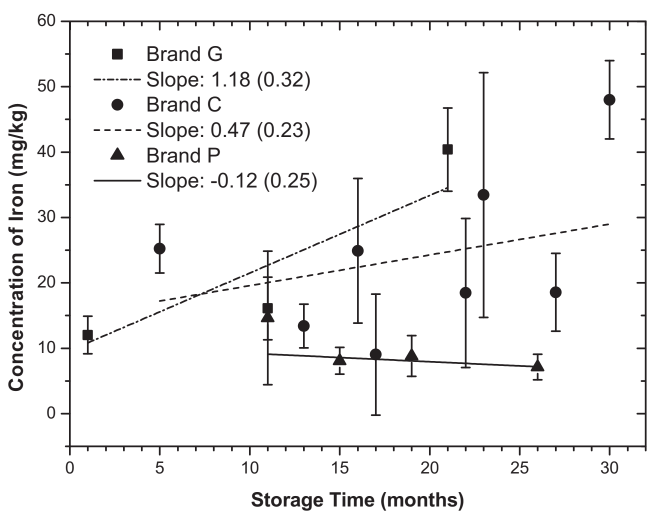

Fig. 4. Concentration of Fe as a function of the storage time evaluated for all brands studied in this work. The full, dashed and dot-dashed lines represent a linear fit to the data from brands P, C and G respectively. The slopes and their uncertainties are shown as well.

A simple linear fitting was carried out for all set of data shown in Fig. 4. An increasing pattern in the concentration of Fe as a function of storage time was observed for brands G and C. Brand P presented a slope compatible to zero, indicating a constant behavior along the storage time. Interestingly, as shown in Fig. 1, brand P is the only one whose can is not rich in iron. However, a definite conclusion is hard to achieve due to the relatively large uncertainties associated to the data. An increase in Fe concentration has already been reported. Indeed, Dantas et al. (2008) evaluated three different types of DRD (Draw and Redraw) cans used for packing tuna fish. The evaluation took into account the color alteration, sensory analysis and the contents of Al, Cr and Fe in the canned tuna fish as a function of the storage period. They observed a significant increase of all elements in a period of 180 days. Our results for Fe seem to support their findings.

4. Conclusions

The elemental composition of canned tuna from three different Brazilian brands was studied through the PIXE technique. The data analysis revealed the presence of Na, Mg, P, Cl, K, Ca, Fe and Zn in all samples. Other elements such as S, Mn, Ni and Cu were absent in some samples. The elemental concentrations of Al, Ti, Cr, Rb, Sr, Mo, Hg and Pb were found to be either compatible or below the LOD of the PIXE system, thus indicating relatively low concentrations of these elements in canned tuna from Brazil. An analysis of different portions of tuna fish in the cans showed that the elemental concentrations are homogeneous inside the cans.

Significant variations in the elemental composition of canned tuna across the brands were observed for most of the elements. These differences may be attributed to different factors like the number of fish species used by the manufacturer and canning. Despite our results suggest a possible influence of these factors on the elemental concentrations observed in this work, it is not possible to draw a definite scenario on this subject due to relatively large uncertainties associated with the data.

Cans were analyzed by the PIXE technique. Two brands studied in this work make use of iron-rich cans, while one of them employs aluminum-rich cans. In any case, elements like Al, Cl, Cr, Mn and Fe were detected in all cans.

A comparison between oil-packed and brine-packed tuna from the same brand shows significant differences for practically all elements. Our results indicate that the elemental concentrations of oil-packed tuna are comparatively higher for most elements than those from brine-packed tuna. Still for the same brand, a comparison of oil-packed tuna fish enclosed in cans and in sachets showed no differences between them. When these results are compared with fresh tuna one observes that fresh tuna is characterized by relatively small concentrations of elements like P, S and Cl.

The analysis of the concentration of Fe in canned tuna as a function of the storage time suggests a slight increase of this element for those brands which employed cans rich in iron. No such trend was observed for the brand which makes use of aluminum-rich cans. This result indicates a possible interaction between the metallic can and its contents when large storage time occurs. Finally, the overall results obtained in this work are in good agreement with previous measurements of canned tuna.

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